Bradbury RH, Callis R, Carr GR, Chen H, Clark E, Feron L, Glossop S, Graham MA, Hattersley M, Jones C, Lamont SG, Ouvry G, Patel A, Patel J, Rabow AA, Roberts CA, Stokes S, Stratton N, Walker GE, Ward L, Whalley D, Whittaker D, Wrigley G, Waring MJ. Optimisation of a series of bivalent triazolopyridazine based bromodomain and extraterminal inhibitors: the discovery of (3R)-4-[2-[4-[1-(3-methoxy-[1,2,4]triazolo[4,3-b]pyridazin-6-yl)-4-piperidyl]phenoxy]ethyl]-1,3-dimethyl-piperazin-2-one (AZD5153). *Journal of Medicinal Chemistry* 2016

Optimisation of a series of bivalent triazolopyridazine based bromodomain and extraterminal inhibitors: the discovery of (3R)-4-[2-[4-[1-(3-methoxy-[1,2,4]triazolo[4,3-b]pyridazin-6-yl)-4-piperidyl]phenoxy]ethyl]-1,3-dimethyl-piperazin-2-one (AZD5153)

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Supporting Information

Contents

Crystallisation and structure of BRD4(1) in complex with 13

E.coli expressed BRD4-(42-169) at a concentration of 10mg/ml was mixed with **13** to a final concentration of 400 μM, 2% DMSO and incubated on ice for 1 hour. Note that due to limited solubility and DMSO tolerance, a molar excess of the compound concentration could not be attained. Low volume sitting drop crystallisation was performed in MRC 2 well plates using a ratio of 2:1 protein : mother liquor and crystals formed in a condition containing 0.2M K_3Citrate and 10% PEG 10k (w/v) at 4 °C. Crystals were cryoprotected in mother liquor supplemented with a further 20% glycerol. Diffraction data was collected on beamline IMCA-CAT 17-ID at the Advanced Photon Source, Argonne, IL, USA using a Dectris Pilatus 6M detector and processed using XDS [Kabsch, W. *Acta Cryst*. **D66**, 125-132 (2010)]. Molecular replacement solution was determined with 2OSS.pdb using Phaser [McCoy, A.J. *et al. J. Appl. Cryst*. **40**, 658-674 (2007)]. The $P2_12_12_1$ cell ($\alpha=\beta=\gamma=90°$, a=41.754Å, b=59.769Å, c=107.151Å) has a dimer in the ASU. Model building was performed using Coot [Emsley, P. *et al*. *Acta Cryst*. **D66**, 486-501 (2010)] and refined using Buster [Bricogne, G. *et al.* Buster version 2.11.6. Cambridge, United Kingdom: Global Phasing Ltd. (2011)]. **13** could easily be modelled into the difference density using ligand restraints generated by Grade [Smart, O.S. *et al*. Grade version 1.2.9. Cambridge, United Kingdom: Global Phasing Ltd. (2011)]. It should be noted that the electron density for the ligand was of sufficient quality to enable it to be built in two alternate and equivalent conformations with a total final occupancy of 1.0. The statistics for the data collection and

refined co-ordinates are given in (Table S1). The final model has been deposited in the protein databank under accession code 5KHM.

Table S1 - X-ray data collection and refinement statistics for BRD4(1) in complex with **13**.

	5KHM (**13**)
Data collection	
Space group	P212121
Cell dimensions	
a, *b*, *c* (Å)	41.75, 59.77, 107.15
A, β, γ (°)	90, 90, 90
Resolution (Å)	53.58 – 1.48 (1.52-1.48) *
R_{svm} or R_{merge}	0.064 (0.918)
I / σI	18.1 (2.5)
Completeness (%)	100 (100)
Redundancy	7.8 (8.0)
Refinement	
Resolution (Å)	53.58 – 1.48
No. reflections	45557
R_{work} / R_{free}	17.9/ 21.0
No. atoms	
Protein	2125
Ligand/ion	35
Water	282
B-factors	
Protein	23.0
Ligand/ion	18.2
Water	33.4
R.m.s. deviations	
Bond lengths (Å)	0.98
Bond angles (°)	0.01

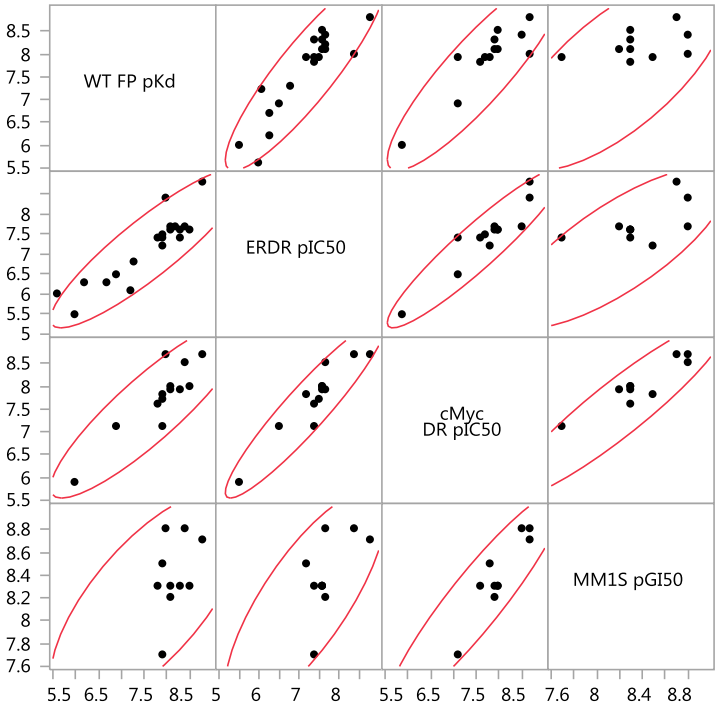
*All structures solved using data from a single crystal. *Values in parentheses are for highest-resolution shell.

Correlations of potency values between BRD4 FP potency, ER downregulation, c-Myc downregulation and growth inhibition.

Table S2 - Correlation coefficients (r^2) between the potency values

	BR4 FP pKi	ERDR pIC50	cMyc DR pIC50	MM1S pGI50
BRD4 FP pKi		0.80	0.78	0.49
ERDR pIC50	0.80		0.88	0.60
cMyc DR pIC50	0.78	0.88		0.85
MM1S pGI50	0.49	0.60	0.85	

Scatterplot matrix



Further discussion

The different cellular context for these cell based readouts needs to be considered. In the end they all serve as surrogate assays for cellular level bromodomain target-engagement (TE) so it is not surprising that these assays correlate for BET inhibitors.

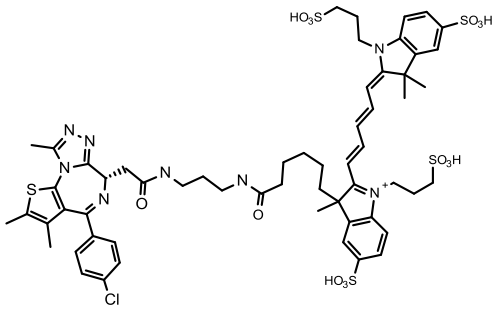
cMyc DR assay was done in multiple myeloma cell line MM1.s. BRD4 controlling Myc expression in MM1.S in bromodomain-dependent manner is well established. Cellular cMyc down-regulation is frequently used to assess BET inhibitor activity in literature.

Our ER DR assay was carried out in MCF7 ER+ breast cancer cell line. BRD4/BRD3 has been shown to be crucial for recruiting WHSC1 to promote ESR1 transcription, again in a bromodomain-

dependent manner (*Cell Res*. **2014**, *24*, 809-819). Thus ER down-regulation may be used to measure BET inhibitor activity and our correlation data enhances this notion.

BRD4 and AR linkage has also been established (*Nature,* **2014**, *510*, 278-82). BRD4 binds directly to the acetylated AR *via* its bromodomains, and BRD4 co-localizes with AR to AR target loci to function as a co-regulator of AR-mediated gene transcription. We believe our AR DR assay measures the nuclear AR level and this readout correlates very well with more established Myc DR assay towards our BET inhibitors and also others such as JQ1 and I-BET762.

Alexa647-labelled probe



2-((6S)-4-(4-chlorophenyl)-2,3,9-trimethyl-6H-thieno[3,2-f][1,2,4]triazolo[4,3-a][1,4]diazepin-6-yl)acetic acid (**2**, 8.0 mg, 0.02 mmol) and tert-butyl(3-aminopropyl)carbamate (3.5 µL, 0.020 mmol) were dissolved in DMF (1.0 mL), then TEA (7.0 µl, 0.050 mmol) was added to give a yellow solution. EDC (4.6 mg, 0.020 mmol) and HOBT (3.7 mg, 0.020 mmol) were added. The reaction was stirred for 16 hours. The reaction was partitioned between EtOAc (10 mL) and satd. $NaHCO_3$ (10 mL). The organic layer was extracted, washed with brine, dried over Na_2SO_4 and stripped to dryness giving crude intermediate tert-butyl (3-(2-((6S)-4-(4-chlorophenyl)-2,3,9-trimethyl-6H-thieno[3,2-f][1,2,4]triazolo[4,3-a][1,4]diazepin-6-yl)acetamido)propyl)carbamate (6.0 mg, 54%). A sample of the intermediate (3.4 mg, 6.10 µmol) was dissolved in DCM (1.0 mL). Trifluoroacetic acid (0.10 mL, 1.3 mmol) was added and stirred for 2 hours. The reaction was stripped to dryness and redissolved in DMSO (1 mL). TEA (4.25 µl, 0.03 mmol) was added, followed by 2-((1E,3E,5E)-5-(3,3-dimethyl-5-sulfo-1-(3-sulfopropyl)indolin-2-ylidene)penta-1,3-dien-1-yl)-3-(6-((2,5-dioxopyrrolidin-1-yl)oxy)-6-oxohexyl)-3-methyl-5-sulfo-1-(3-sulfopropyl)-3H-indol-1-ium (5.8 mg, 6.1 µmol). The reaction was stirred for 16 hours. The mixture was purified directly by preperative HPLC, eluting with 0-50%

MeCN in water (NH_3 modifier). This delivered the desired product 3-(6-((3-(2-((6S)-4-(4-chlorophenyl)-2,3,9-trimethyl-6H-thieno[3,2-f][1,2,4]triazolo[4,3-a][1,4]diazepin-6-yl)acetamido)propyl)amino)-6-oxohexyl)-2-((1E,3E,5E)-5-(3,3-dimethyl-5-sulfo-1-(3-sulfopropyl)indolin-2-ylidene)penta-1,3-dien-1-yl)-3-methyl-5-sulfo-1-(3-sulfopropyl)-3H-indol-1-ium (2.5 mg, 32%) as a blue gum.

1H NMR (300 MHz, CD_3OD) δ 0.6 (br s, 1H), 0.9 (br d, *J* = 5.5 Hz, 2H), 1.2 - 1.3 (m, 3H), 1.3 (t, *J* = 7.3 Hz, 6H), 1.4 - 1.5 (m, 2H), 1.6 - 1.8 (m, 13H), 2.0 (t, *J* = 7.3 Hz, 2H), 2.2 (br d, *J* = 11.3 Hz, 5H), 2.4 (s, 3H), 2.7 (d, *J* = 1.3 Hz, 3H), 2.9 - 3.0 (m, 4H), 3.1 - 3.3 (m, 6H), 3.3 - 3.4 (m, 1H), 4.3 (br s, 4H), 4.6 (dd, *J* = 8.7, 5.7 Hz, 1H), 6.5 (br d, *J* = 13.6 Hz, 2H), 6.7 (br t, *J* =12.8 Hz, 1H), 7.3 - 7.5 (m, 6H), 7.8 - 7.9 (m, 4H), 8.2 - 8.4 (m, 2H). Purity 94% by LC-MS, *m/z*: ES^+ $[M+H]^+$ 1297.3